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Comparison of phenotypic and genetic traits of ESBL-producing UPEC strains causing recurrent or single episode UTI in postmenopausal women

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Abstract

Background Recurrent urinary tract infections (rUTIs) occur in over 20% of patients, with postmenopausal women (over 50 years old) carrying the highest risk for recurrence compared to younger women. Virulence factors such as type 1 fimbriae adhesin FimH, the outer membrane protease OmpT, and the secreted pore-forming toxin α-hemolysin (HlyA) have been shown to support the formation of intracellular bacterial communities (IBCs) within bladder epithelial cells (BECs), facilitating persistence. This study aims to characterize the virulence expression and intracellular persistence of ESBL-producing uropathogenic *E. coli* (E-UPEC) strains isolated from postmenopausal women with recurrent or single episode infections.

Methods Study strains included 72 E-UPEC strains collected from patients (36 recurrent; 36 single episode) with a confirmed UTI diagnosis and control UPEC strains (CFT073 and UTI89). Patient demographics and clinical course were collected. Presence of *hlyA*, *ompT*, and *fimH* genes were confirmed by colony PCR, and qRT-PCR was performed using extracted RNA from a subset of 18 strains (12 recurrent; 6 single episode) grown in Luria-Bertani media and isolated from infected BECs to characterize gene expression. Bladder cell line 5637 was infected with study strains at MOI 15 for 2 h, treated with amikacin for 2 h to remove extracellular bacteria, then lysed to enumerate intracellular CFU counts.

Results No differences in clinical characteristics between patient groups were observed. Overall prevalence of *fimH*, *ompT*, and *hlyA* was 99% (71/72), 82% (59/72), and 26% (19/72) respectively; presence of all three genes did not differ between recurrent and single-episode strains. Notably, all recurrent strains had significantly more intracellular CFUs compared to single episode strains (median 16,248 CFU/mL vs. 4,118 CFU/mL, $p=0.018$). Intracellular expression *ompT* was significantly increased ($p=0.0312$) in the recurrent group compared to LB media, while *fimH* was significantly decreased ($p=0.0365$) in the single episode group compared to expression in LB media.

Conclusion Our findings indicate strain-specific ability to persist inside BECs with the recurrent strains exhibiting increased *ompT* expression inside BECs and higher intracellular bacterial burden compared to strains causing single

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episode UTI. These results emphasize the potential microbial contributions to recurrence in postmenopausal women and warrant future investigations on the impact of antibiotic therapy and host response on IBC-supportive UPEC virulence.

Keywords Recurrent UTIs, UPEC, Postmenopausal women, Intracellular bacterial communities

Background

Urinary tract infection (UTI) is one of the most common infections worldwide and responsible for over 11 million outpatient visits and costing over 4 billion dollars annually in the United States [1–3]. Women experience the highest risk for contracting UTIs with over 20% of female patients experiencing recurrence [4, 5]. Among those with recurrent UTIs, the subpopulations most affected are women in postmenopausal age and when extended spectrum beta-lactamase (ESBL) producing uropathogenic *Escherichia coli* (UPEC) is the causative pathogen [3, 6–8]. In addition to carrying a multidrug-resistant phenotype, UPEC strains can form intracellular bacterial communities (IBCs) which are biofilm-like structures within bladder epithelial cells (BECs) that confer protection of the bacteria from antibiotic and immune cell exposure [9–12]. These IBCs go through a cycle of reinfection of surrounding BECs, allowing the bacteria to spread deeper within the bladder tissue of postmenopausal women [4, 9]. When left untreated or incompletely treated, these bladder infections, also known as cystitis, can progress to infections of the kidney (pyelonephritis) and urosepsis may ensue [9, 13]. UPEC virulence factors that have been identified to support IBC formation within BECs include type 1 fimbrial adhesin FimH which facilitates attachment to BECs, pore-forming toxin α-hemolysin (HlyA) which allows UPEC to escape lysosomal compartments within BECs, and outer membrane protease OmpT which can degrade host antimicrobial peptides and contributes to intracellular biofilm formation [9, 14–16]. The *fimH* gene is only encoded on the chromosome, while *hlyA* and *ompT* genes can both be encoded on the chromosome as well as on plasmids [17–20]. Overall prevalence of *fimH*, *hlyA*, and *ompT* ranges from 86 to 100%, 21–47%, 67–94%, respectively [21–25]. However, the prevalence of these genes among UPEC strains isolated from postmenopausal women and particularly among those with recurrence is unknown. Significant reduction in IBCs has been shown in both in vitro and in vivo models of bladder infection with UPEC strains upon deletion of *fimH*, *hlyA*, and/or *ompT*, supporting their role in UPEC pathogenesis [14–16, 19, 26]. While patient factors such as comorbid conditions and genetic polymorphisms as contributing factors to recurrence have been well described, whether microbial characteristics differ between UPEC strains causing recurrent or single episode infections among postmenopausal women has not been well studied [7, 27, 28]. Therefore, we hypothesized that UPEC isolates from postmenopausal women with recurrent episodes are better able to survive inside of BECs than single episode strains due to robust virulence factor expression supporting IBC formation during infection. In this study, we examined the clinical characteristics of postmenopausal women who experienced UTI caused by ESBL-producing UPEC strains and profiled the expression of *fimH*, *hlyA*, and *ompT* as well as the in vitro intracellular persistence in BECs. Our results indicate marked differences in virulence factor expression and intracellular persistence between strains causing single versus recurrent episodes of UTI. This study emphasizes the importance of investigating the contribution of microbial virulence to recurrent UTIs.

Methods

Study design

This study was conducted at a community-teaching hospital on patients hospitalized during 2014–2021 under an IRB-approved protocol #HS-17-00943. Informed consent was waived since no interventions were made. Microbiology reports were reviewed to identify patients who had a urine culture positive for ESBL-producing UPEC strain. ESBL phenotype was confirmed by the clinical microbiology laboratory by disk diffusion using ceftazidime and ceftazidime/clavulanic acid discs in accordance with CLSI guidelines [29]. Electronic medical records were reviewed to screen for eligibility based on the following inclusion criteria: age 50 years or older (postmenopause), female sex, diagnosis of UTI based on symptomology, urinalysis, and/or discharge diagnosis, and the bacteria strain had been saved as part of a longitudinal surveillance program to evaluate antimicrobial resistance trends at the institution. Single episode was defined as the absence of either a UTI diagnosis or positive urinalysis within 6 months of the "index" episode while recurrence was defined as two symptomatic episodes in 6 months or 3 symptomatic episodes in 12 months. Episodes greater than 30 days apart were defined as separate UTI episodes. Patients were excluded if younger than 50 years old, male, or without UTI diagnosis. The following demographic and clinical characteristics were obtained from the medical records: age, ethnicity, UTI diagnosis, history of UTI within 12 months, comorbid conditions such as diabetes, dementia, kidney disease, immunocompromised status, and functional quadriplegia, presence of a chronic Foley catheter, antibiotic prescribed, and discharge disposition

[28, 30, 31]. Saved bacteria strains were subjected to molecular and phenotypic analysis. All collected data was managed with the Research Electronic Data Capture (REDCap) software hosted at the University of Southern California, a secure web-based application designed to support data capture for research studies [32]. A subset of the UPEC strains matched by age were selected to represent recurrent and single episode strains in a 2:1 ratio to evaluate intracellular survival using an in vitro bladder infection model.

Presence of IBC-supportive genes

Polymerase chain reaction (PCR) was performed to identify the presence of IBC-supportive genes *fimH*, *hlyA*, and *ompT* in ESBL-UPEC isolated from study patients. These IBC-supportive genes were chosen due to significant decreases in intracellular CFUs of deletion mutants observed in both cell line and mouse models of UTI infection [14, 16, 26, 33]. Colony PCR using a single colony of each index strain from CHROMagar plates incubated at 37 °C overnight was used with Green GoTaq mastermix (Promega) and 250 nM primer concentration per the manufacturer's instructions to perform an initial screen for the presence of *fimH*, *hlyA*, and *ompT* in the genome. If genes were not detected by colony PCR, colonies of bacteria from incubated CHROMagar plates were grown overnight at 37 °C, 250 rpm in LB Lennox broth for genomic DNA extraction using the QIAmp Mini extraction kit per the manufacturer's instructions. Extracted genomic DNA (250 ng) was used with 250 nM primer concentration to perform PCR using the Green GoTaq mastermix (Promega) in 20 uL reaction mix per the manufacturer's instructions to verify absence of the genes. UPEC reference strain UTI89 (cystitis strain) was used as a positive control given that it contains all three genes [15, 33, 34]. A previously sequenced UPEC strain with confirmed absence of *ompT* and *hlyA* was used as a negative control. The *cysG* gene was used as a reference, and the PCR products were visualized on a 1.5% agarose gel using gel electrophoresis (Bio Rad) [35]. Primer sequences and conditions are listed in Supplemental Table 1.

Intracellular CFU assay

UPEC strains were selected to represent those causing recurrent and single episode respectively as well as presence or absence of IBC-supportive genes (*hlyA* and *ompT*; *fimH* was near universally present) to examine their propensity for intracellular survival and persistence. The assay was performed following published protocol with modifications [10]. Briefly, human bladder epithelial cell line 5637 (ATCC HTB9) was selected for in vitro modeling of UTI in accordance with previous studies, and cultivated in RPMI + 10% FBS at 37 °C, 5% CO_2 for up to nine passages [14, 16, 26, 33, 36]. Colonies of bacteria were incubated at 37 °C, 250 rpm in LB Lennox broth for 12–14 h, washed twice in chilled DPBS, and resuspended in RPMI media without fetal bovine serum (Gibco). 1.3×10^5 of BECs in RPMI + 10% FBS were seeded in 48-well plates 16 h prior to the start of the experiment. At the start of the experiments, monolayer was confirmed by light microscope and cells were washed once in warmed DPBS. Bacteria in RPMI media were adjusted to OD600 0.05, diluted 1/20 in 5 mL of RPMI + 10% FBS, then 400 uL of diluted bacteria was added to 48-well plate of washed BECs (final MOI 15). Infection was synchronized by centrifugation at 600 xg, 25 C for 5 minutes then incubated at 37 C, 5% CO2 for 2 h. Infecting dilution was also plated on LB agar in duplicate for CFU counts to confirm MOI. Extracellular bacteria were washed three times using warmed DPBS and 400 uL of RPMI + 10% FBS + 450 ug/mL amikacin was added to the wells and incubated at 37° C, 5% CO2 for 2 h to kill remaining extracellular bacteria. Amikacin was used instead of gentamicin given high gentamicin resistance observed in the clinical strains and low cell penetration [37]. BECs were then washed twice using DPBS, lysed using 0.1% Triton X-100, and diluted and plated on LB agar in duplicate. Plates were incubated at 37° C overnight and counted. Intracellular counts were normalized to initial CFU at the start of infection. UTI89 and CFT073 were used as positive controls as their intracellular persistence was previously described [14, 26]. The experiment was performed in six technical replicates and biological duplicates per strain.

Expression of IBC-supportive genes in LB and during in vitro infection

To assess expression of IBC-supportive genes in Luria-Bertani (LB) media, bacteria were grown in LB Lennox overnight at 37° C, 250 rpm, diluted then grown to mid-log phase (OD600 0.3) then extracted using the Purelink Mini RNA extraction kit per manufacturer instructions (Invitrogen). To assess intracellular expression of IBC-supportive genes, the intracellular CFU assay was performed above with modifications. 2×10^6 BECs were seeded in a 6 well plate and infected approximately 16 h later at MOI 50 to ensure sufficient yield of intracellular bacterial RNA [38–40]. After the incubation with amikacin, wells were washed and lysed with lysis buffer, then extracted using the Purelink Mini RNA extraction kit per manufacturer instructions (Invitrogen). All RNA was treated with Turbo DNAse and cleaned using RNA Clean and Concentrate (Zymo). Purity and concentration of RNA was determined using threshold 260/280 and 260/230 values of 2.0 and above obtained using Nanodrop (ThermoFisher). Removal of genomic DNA contamination was verified using qPCR consisting of a

10 uL reaction with 18.75 ng/uL RNA (three times final concentration) and 300 nM *cysG* primers. A total of 1 ug RNA in addition to iScript mastermix (BioRad) was used to create cDNA, which was subsequently diluted to a final concentration of 6.25 ng/uL in nuclease free water. 25 ng of cDNA was used in a 10 uL reaction with SsoAdvanced SYBER mastermix (Biorad) and 300 nM final primer concentration. The *cysG* gene was used as a reference, and the ΔΔCT method was used for relative expression analysis using UPEC reference strains UTI89 (cystitis strain) and CFT073 (pyelonephritis strain) as a positive controls given that they both contain all three genes [14, 15, 33–35]. The experiments were performed in biological duplicates.

Statistical analysis

Chi-squared test, Fisher's exact test, and descriptive statistics on clinical data were performed using SPSS Statistics (IBM, version 29). Fisher's exact test, Mann-Whitney test, paired t-test, and correlation analysis on experiemental data were performed using software GraphPad Prism 10 (Graphpad, San Diego, CA, version 10.4.1).

Results

Patient characteristics

A total of 72 patients were evaluated for this study. Postmenopausal women with at least one UTI diagnosis were grouped based on a history of single or recurrent episodes and were compared with respect to demographics and comorbid conditions particularly those known to predispose to UTI development (Table 1). The age of the study patients ranged from 50 to 95 years old with an overall median age of 73.5 years. Patients in the recurrent group were slightly older compared to those with single episode (median age of 78 vs. 72 years). The majority of the patients identified as White (50.0%, 36/72) followed by Hispanic (29.2%, 21/72). The majority of the patients were diagnosed with cystitis (84.7%, 61/72), followed by pyelonephritis (8.3%, 6/72) and urosepsis (2.8%, 2/72). Three patients (Other, 4.2%) had abnormal urinalysis but no mention of a diagnosis in the medical records. In the recurrent arm, the median number of previous episodes within the last 12 months was 3 (IQR 2, 4). The overall prevalence of comorbid conditions among study patients were 25% (18/72) for diabetes, 23.6% (17/72) kidney disease, 20.8% (15/72) immunocompromised status, 20.8% (15/72) dementia, 4.2% (3/72) functional quadrepelgia, and 4.2% (3/72) with a chronic urinary catheter. The prevalence of each comorbid condition was similar between study groups except for diabetes where there were twice as many patients in the recurrent compared to the single episode group (33.3%, 12/36 vs. 16.7%, 6/36); all three patients with chronic urinary catheters were in the recurrent arm. Most patients were admitted to the hospital (66.7%, 48/72) then discharged home after receiving antibiotic therapy (55.6%, 40/72). Ertapenem was the most frequently prescribed antibiotic (37.5%, 27/72), followed by ceftriaxone (33.3%, 24/72) and nitrofurantoin (22.2%, 16/72). Antibiotic therapies were similar between the study groups.

Prevalence of IBC-supportive genes

We investigated the molecular determinants of IBC formation using PCR to compare the prevalence of *fimH*, *hlyA*, and *ompT* among strains in each study group. The *fimH* and *ompT* genes were observed to be the most prevalent among the 72 UPEC strains isolated from postmenopausal women with 98.6% (71/72) and 81.9% (59/72) of strains possessing the genes respectively, whereas the *hlyA* gene was present in 26.4% (19/72) of strains. The majority of strains had both *fimH* and *ompT* (55.6%, 40/72), with a smaller subset of strains carrying all three genes (25.0%, 18/72), or only *fimH* (16.7%, 12/72). The prevalence of all three genes did not differ between strains from patients with recurrent or single episode UTI (Fig. 1; Fisher's exact test, *fimH p* value > 0.999, *ompT p* value = 0.5414, *hlyA p* value = 0.1073). In the single episode group, one strain possessed only *ompT*, and another strain had only *hlyA* and *fimH*.

Intracellular persistence of UPEC strains

We next sought to characterize the IBC phenotype between recurrent and single episode strains using a modified intracellular CFU assay. Twelve strains from recurrent patients and six strains from patients with single episodes were selected for further investigation based on the presence of *ompT* with or without the presence of *hlyA* to represent the population of isolated strains, and to enrich for the recurrent group (study group of interest) for the detection of potential differences between the groups. Three out of the twelve selected strains from the recurrent arm as well as four out of six selected strains from the single episode arm also contained the *hlyA* gene in addition to *fimH* and *ompT* genes. Overall, recurrent strains showed four-fold greater intracellular CFU counts compared to single-episode strains following 5 h of infection (median 16,248 CFU/mL vs. 4,118 CFU/mL, $p = 0.0182$) (Fig. 2). The range of intracellular CFU counts also varied widely between recurrent and single episode strains (7,172–129,300 average CFU/mL versus 1,848–33,258 CFU/mL), indicating differential ability to persist intracellularly within bladder epithelial cells.

Expression of IBC-supportive genes during infection and laboratory conditions

Since we observed differential intracellular persistence between recurrent and single episode strains, we investigated whether the expression of IBC-supportive

Table 1 Study participant characteristics

	All Patients (*n* = 72)	Recurrent Patients (*n* = 36)	Single episode Patients (*n* = 36)	*P* value[a]
Age (y, median (IQR))	73.5 (64, 83.5)	78 (68.25, 88.5)	72 (60.5, 80)	0.239
Race (%)				
White	36 (50.0)	17 (47.2)	19 (52.8)	0.637
Black	6 (8.3)	4 (11.1)	2 (5.6)	0.674
Hispanic	21 (29.2)	13 (36.1)	8 (22.2)	0.195
Asian	3 (4.2)	0	3 (8.3)	0.239
Other	6 (8.3)	2 (5.6)	4 (11.1)	0.674
UTI Diagnosis (%)				
Urinary tract infection	61 (84.7)	31 (86.1)	30 (83.3)	0.743
Pyelonephritis	6 (8.3)	2 (5.6)	4 (11.1)	0.674
Urosepsis	2 (2.8)	1 (2.8)	1 (2.8)	1.000
Other	3 (4.2)	2 (5.6)	1 (2.8)	1.000
History of UTI (%)	26 (36.1)	26 (72.2)	N/A	
No. of episodes w/in 12 mon (median (IQR))	1 (0, 3)	3 (2, 4)	N/A	
Comorbid Conditions (%)				
Diabetes	18 (25.0)	12 (33.3)	6 (16.7)	0.173
Immunocompromised	15 (20.8)	6 (16.7)	9 (25.0)	0.563
Kidney Disease	17 (23.6)	11 (30.6)	6 (16.7)	0.267
Chronic foley catheter	3 (4.2)	3 (8.3)	0	0.239
Dementia	15 (20.8)	9 (25.0)	6 (16.7)	0.563
Functional quadriplegia	3 (4.2)	1 (2.8)	2 (5.6)	1.000
Admission (%)				
ED	21 (29.1)	9 (25.0)	12 (33.3)	0.605
Hospital	48 (66.7)	24 (66.7)	24 (66.7)	1.000
Other	3 (4.2)	3 (8.3)	0	0.239
Discharge Disposition (%)				
Home	40 (55.6)	19 (52.8)	21 (58.3)	0.635
Skilled nursing facility	25 (34.7)	14 (38.9)	11 (30.6)	0.458
Deceased	3 (4.2)	0	3 (8.3)	0.239
Other	4 (5.6)	3 (8.3)	1 (2.8)	0.614
No. of Antibiotics Prescribed (median (IQR))	2 (1, 2)	2 (1, 2)	2 (1, 2)	
Antibiotic prescribed (n (%))				
Ertapenem	27 (37.5)	15 (41.7)	12 (33.3)	0.627
Meropenem	11 (15.3)	5 (13.9)	6 (16.7)	0.865
Trimethoprim-Sulfamethoxazole	7 (9.7)	2 (5.6)	5 (13.9)	0.460
Nitrofurantoin	16 (22.2)	11 (30.6)	5 (13.9)	0.182
Fosfomycin	1 (1.4)	1 (2.8)	0	0.532
Ciprofloxacin	6 (8.3)	2 (5.6)	4 (11.1)	0.643
Piperacillin-Tazobactam	7 (9.7)	3 (8.3)	4 (11.1)	0.842
Amoxicillin-Clavulanate	2 (2.8)	1 (2.8)	1 (2.8)	0.840
Cephalexin	3 (4.2)	2 (5.6)	1 (2.8)	0.742
Cefdinir	1 (1.4)	1 (2.8)	0	0.532
Cefuroxime	3 (4.2)	2 (5.6)	1 (2.8)	0.742
Ceftriaxone	24 (33.3)	10 (27.8)	14 (38.9)	0.584
Gentamicin	1 (1.4)	0	1 (2.8)	0.358

[a] Mann-Whitney t-test performed for continuous variables and Chi-squared or Fisher's exact test performed for categorical variables as appropriate comparing recurrent and single-episode patients

virulence genes varied between recurrent and single episode strains during infection of BECs and when grown in laboratory media. Notably, we observed gene expression differences when grown in LB media compared to during infection in BECs and between strains causing single or recurrent episodes. When strains were grown in LB media, expression of *fimH* and *ompT* was similar between the two groups, $p = 0.7503$ and 0.8201 respectively (Supplemental Fig. 1). However, when the gene expression data was compared between intracellular

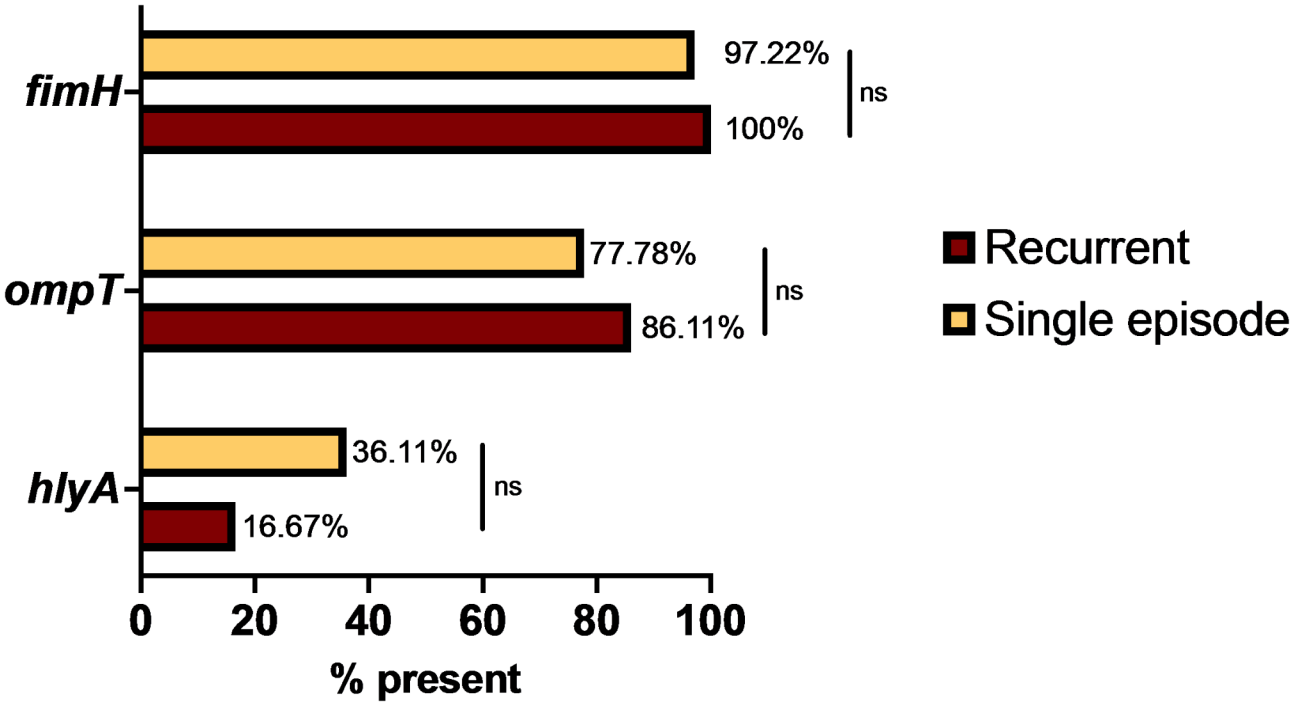

Fig. 1 Presence/absence of virulence genes in UPEC strains isolated from 72 postmenopausal women. PCR was performed to amplify genes, then visualized using gel electrophoresis. UTI89 used as positive control; *cysG* used as the reference gene. Fisher's exact test performed to compare the prevalence of each gene in recurrent and single episode strains (*fimHp* value > 0.999, *ompTp* value = 0.5414, *hlyAp* value = 0.1073)

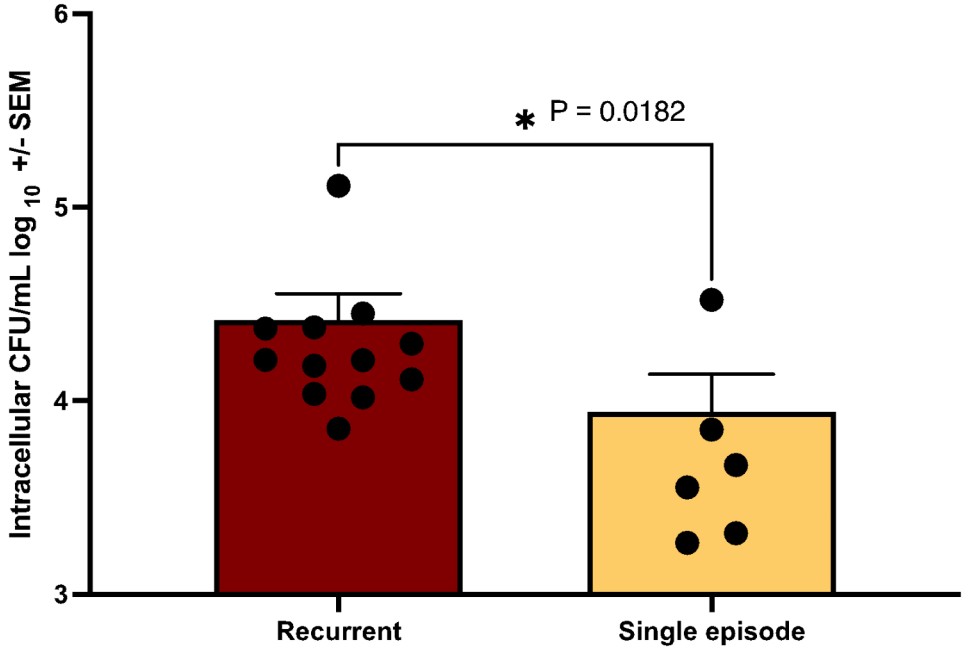

Fig. 2 Comparison of intracellular CFU counts of clinical UPEC from recurrent and single episode patients. 12 recurrent strains, and 6 single episode strains were used to infect bladder cell line 5637 at MOI 15 for two hours. Extracellular bacteria were washed away and cells treated with 450 ug/mL amikacin for two hours, then lysed and counted. Mann-Whitney test performed

populations of bacteria and growth in LB media, *ompT* expression was significantly increased in the recurrent group (Fig. 3, $p = 0.0312$), supporting its role in virulence. Interestingly, among the single episode strains, *fimH* was significantly decreased in the intracellular population compared to growth in LB media (Fig. 3, $p = 0.0365$) while recurrent strains did not differ in expression between LB and intracellular environments (Fig. 3). No difference in

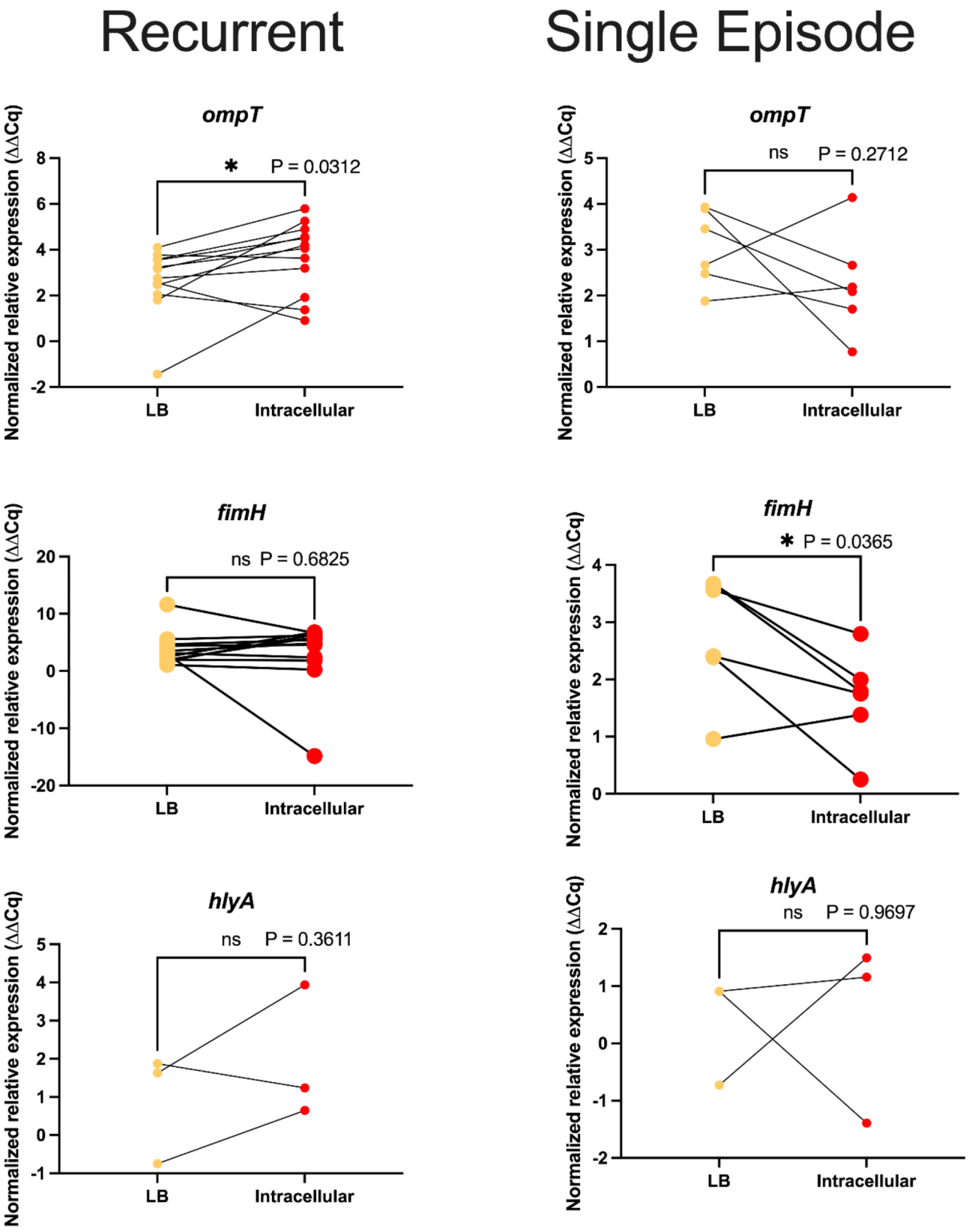

Fig. 3 Normalized *ompT*, *fimH*, and *hlyA* expression in LB media and during BEC infection (ΔΔCq). Bacteria incubated to mid-log phase in LB for RNA extraction; BEC infection performed at MOI 50 as described for intracellular RNA extraction. Normalized to *cysG* and control strains, UTI89 and CFT073. Paired t-test performed

hlyA expression was observed between LB and infection for both groups. A trend towards higher intracellular expression of *fimH* and *ompT* was observed in recurrent strains when directly compared to that in single episode strains, with median ΔΔCq values 3- and 2-fold higher, $p = 0.0529$ and 0.0831, respectively (Supplemental Fig. 1). Taken together, these results suggest strain differences between those causing recurrent versus single episodes of UTI in postmenopausal women in both the intracellular expression of IBC-supportive genes and the intracellular persistence phenotype.

Exploratory correlation analysis between intracellular CFUs and intracellular expression of *ompT* and *fimH*

Given the observed increase in intracellular gene expression, we performed exploratory correlation analysis between intracellular gene expression and intracellular CFUs produced by all 18 strains using Spearman correlation. Intracellular expression of *ompT* and *fimH* displayed a modest positive correlation with intracellular CFUs (Supplemental Fig. 2A; $r = 0.23$, 0.39 (p values = 0.367 and 0.247)). However, removal of the recurrent strain outlier yielded a stronger correlation with only *fimH* expression as statistically significant (Supplemental Fig. 2B; $r = 0.42$, 0.53 (p values = 0.095 and 0.031)). These preliminary results suggest the potential contribution of both *ompT* and *fimH* to intracellular survival of UPEC in bladder epithelial cells.

Discussion

Recurrent UTIs have been attributed in part to the development of persistent bacterial reservoirs from invading UPEC forming intracellular bacterial communities (IBCs), as the cycle of IBC formation facilitates spread of the bacteria deep into the bladder tissue [12, 41]. This phenomenon has been observed in the bladders of postmenopausal women, who have been previously identified as high risk for developing recurrent UTIs [4]. Although observed within the bladders of postmenopausal women, the role of microbial determinants in IBC formation in this high risk population remains unclear. This study sought to examine the clinical characteristics of postmenopausal women who experienced recurrent versus single episode UTIs caused by ESBL-producing UPEC strains and compare the virulence gene expression and differential intracellular persistence of the infected strains. Patient factors known to increase risk of recurrence such as immunocompromised status and kidney disease were similar between groups, with twice as many diabetic patients in the recurrent arm though the difference was not statistically significant [3, 28, 30, 31].

With respect to microbial characteristics, the IBC-supportive genes *fimH*, and *ompT* were both highly prevalent across study strains, irrespective of recurrent or single episode UTI. The prevalence of each gene was consistent with published literature [21, 24, 25, 42–44]. Notably, we have shown that expression of *ompT* by the recurrent strains was significantly increased during infection compared to when grown in standard laboratory media. Additionally, a trend towards higher expression was observed in recurrent strains compared to single episode strains but will need to be confirmed with a larger sample size. These results highlighted the potential importance of OmpT in the intracellular environment, which is consistent with previous literature linking OmpT to biofilm formation [24, 45]. As the IBC structure has previously been observed to be biofilm-like, the increase in intracellular *ompT* expression may support the formation and/or maintenance of IBCs within BECs [11, 46]. Interestingly, type 1 fimbrial adhesin FimH expression was increased during in vitro infection in BECs among recurrent strains compared to expression during infection with single episode strains, and was significantly decreased in single episode strains during infection compared to expression in LB media. These results suggest that the role of FimH in the intracellular process of IBC formation may be strain dependent and relies on the microbial production of FimH which can be regulated by environmental cues such as osmolarity [47, 48]. Previous studies have also shown in both UPEC and *K. pneumoniae* that mutations in the FimH protein directly impact IBC formation and bladder colonization [33, 49]. Mutations supporting increased attachment and IBC formation may work in concert with other virulence factors to persist in the bladder tissue. When directly comparing intracellular expression of *ompT* and *fimH* between recurrent and single episode strains, there was no significant difference between the groups although a trend towards increased expression in the recurrent group was observed. Notably, significantly greater intracellular CFUs were found with recurrent strains compared to single episode strains. This study is the first to describe a significant difference in intracellular persistence of ESBL-producing UPEC isolated from postmenopausal women in association with UTI recurrence. Differential ability to persist intracellularly may be attributed to adaptive changes in virulence gene expression and production that develop in vivo. A modest positive correlation was observed between intracellular CFUs and intracellular expression of *ompT* and *fimH* for all 18 strains though not statistically significant, and show potential contributions of intracellular gene expression changes to adaptation in the bladder environment. For example, differential production of capsule production, alterations in metabolism, and the accumulation of various mutations supporting overall virulence, resistance, and metabolism changes over time has been observed in *K. pneumoniae* strains which successfully persisted in the bladder to cause chronic infection [50,

51]. Virulence gene expression changes in UPEC strains may support intracellular survival and adaptation to their particular host. Previous studies investigating virulence and metabolic gene expression in UPEC isolated from females with active UTI showed large variations in *fimH* expression in human urine as well as expression differences between voided urine and active infection [52, 53]. Urine composition and microbiome can vary between pre- and post-menopausal women, and between women in the same life stage [54–57]. Adaptation to each host may provide evolutionary and fitness benefits to infecting UPEC strains, as expression profiles vary significantly between laboratory media and urine as well as between patients given the variation in available nutrients and metabolites [52, 53, 57, 58]. Further studies with larger sample sizes are needed to confirm the direct contribution of OmpT and FimH, and to elucidate whether UPEC virulence factors change over time to better support chronic infection and persistence in the bladders of postmenopausal women.

The primary strength of this study lies in the investigation of the molecular characteristics of the bacteria causing the infections to complement the clinical characteristics of the patients experiencing recurrent UTIs. We are the first to show that strains causing recurrent infections in postmenopausal women have differential abilities to form IBCs compared to strains causing single episodes. The intracellular CFU counts for UPEC strain CFT073 were also consistent with previous studies using a bladder epithelial cell monolayer model, validating our experimental approach [14, 16, 37]. This work emphasizes the importance of using molecular determinants such as expression of bacterial protease OmpT in addition to patient factors in devising therapeutic strategies targeting IBC formation to prevent recurrent infection.

Several limitations to this study are notable. This retrospective study had a small sample size and the patients were selected from a single hospital site, although the patient demographics match a recently published large single-site epidemiology study on 374,171 women experiencing recurrent UTIs [3]. Patients in the single episode study group were identified based on single site visit for UTIs on medical records; however, it is possible that those patients could visit other clinic sites for UTI diagnosis and treatment. Infections were performed in an in vitro static model of the bladder using a monolayer of BECs which only consists on one layer of epithelium. We plan to investigate the development of IBCs using strains from these recurrent UTI patients in a 3D model of the bladder using primary human bladder epithelial cells to examine the adaptation of identified UPEC virulence factors over time in bladder tissue.

Conclusions

ESBL-UPEC strains causing recurrent UTIs in postmenopausal women exhibit differential expression of IBC-supportive virulence factors and intracellular persistence phenotypes as demonstrated in this study. Future investigations will explore the antivirulence potential of current and new treatments on the expression of IBC-supportive virulence factors and their efficacy in the clearance of IBCs generated by ESBL-UPEC from postmenopausal women.

Abbreviations

UTI	Urinary tract infection
UPEC	Uropathogenic Escherichia coli
ESBL	Extended spectrum beta-lactamase
BECs	Bladder epithelial cells
IBCs	Intracellular bacterial communities
CFUs	Colony forming units

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12941-025-00779-7.

Supplemental Fig. 1. Normalized ***ompT*** and ***fimH*** expression in (A) LB media and (B) during BEC infection (ΔΔCq). Bacteria incubated to mid-log phase in LB for RNA extraction; BEC infection performed at MOI 50 as described for intracellular RNA extraction. Normalized to ***cysG*** and control strains, UTI89 and CFT073. Mann-Whitney t-test performed.

Supplemental Table 1. PCR Primers and conditions.

Supplemental Fig. 2. Spearman correlation matrix between average intracellular CFUs and average intracellular expression of ***ompT*** and ***fimH*** for (A) all strains and (B) without recurrent strain outlier. Spearman r value listed for each comparison with blue as positive correlation and red as negative correlation. ***P*** values less than 0.05 are starred.

Supplementary Material 4

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Bacterial strain UTI89, isolated from a patient diagnosed with cystitis, was graciously provided by the laboratory of Dr. Scott Hultgren. Human bladder epithelial cell line 5637 (HTB9) as well as strain CFT073 (ATCC 700928), isolated from a patient diagnosed with pyelonephritis, were obtained from the American Type Culture Collection (ATCC).

Author contributions

M.K. was responsible for the conceptualization, execution of experiments, data analysis, preparation of all figures and tables, and writing of the manuscript. P. J. assisted in the conceptualization, data analysis, and writing of the manuscript. A.W.B. was responsible for the funding, conceptualization, data analysis, and writing of the manuscript. All authors read, reviewed, and approved the manuscript for publication.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Electronic medical record data was collected under IRB approval (HS-17-00943). Informed consent was waived as no interventions were made.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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